

## Dynamically polarized samples for neutron protein crystallography at the Spallation Neutron Source

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**Abstract.** To prepare for the next generation neutron scattering instruments for the planned second target station at the Spallation Neutron Source (SNS) and to broaden the scientific impact of neutron protein crystallography at the Oak Ridge National Laboratory, we have recently ramped up our efforts to develop a dynamically polarized target for neutron protein crystallography at the SNS. Proteins contain a large amount of hydrogen which contributes to incoherent diffraction background and limits the sensitivity of neutron protein crystallography. This incoherent background can be suppressed by using polarized neutron diffraction, which in the same time also improves the coherent diffraction signal. Our plan is to develop a custom Dynamic Nuclear Polarization (DNP) setup tailored to neutron protein diffraction instruments. Protein crystals will be polarized at a magnetic field of 5 T and temperatures of below 1 K. After the dynamic polarization process, the sample will be brought to a frozen-spin mode in a 0.5 T holding field and at temperatures below 100 mK. In a parallel effort, we are also investigating various ways of incorporating polarization agents needed for DNP, such as site specific spin labels, into protein crystals.

### 1. Introduction

One of the difficulties in neutron protein crystallography is the incoherent hydrogen scattering background. One way to reduce this background is sample perdeuteration [1]. However, perdeuteration is still expensive and growing deuterated crystals is not always as easy as compared to nondeuterated ones. An alternative technique is to use polarized neutron scattering from dynamically polarized proteins [2–5]. The technique exploits the high polarization-dependent neutron scattering cross-section of hydrogen. When proton spins are aligned parallel to those of the scattering neutrons, the large, 80 barn incoherent hydrogen scattering cross-section is reduced to zero. In the meantime, the 1.8 barn coherent cross-section is increased to 14.7 barns (Fig 1). The main challenge to polarized neutron protein crystallography is to achieve a high degree of hydrogen or proton polarization. For proteins and other adiabatic samples, the only viable technique today is Dynamic Nuclear Polarization (DNP). A typical DNP setup for high nuclear polarization requires a magnet field of about 2.5 T – 5 T and a sample temperature of 1 K or lower. Under these conditions, the static hydrogen nuclear polarization is only a small fraction of a percent, which is negligible for neutron scattering. In the meantime and under the same conditions, electron spins are close to being fully polarized. DNP takes advantage of the fact that electron spins are readily polarized. A small amount of paramagnetic impurities (on the order of  $\sim 10^{19}$  electron spins per millilitre) are introduced into the sample. Microwave radiation is then used to drive polarization transfer from unpaired electron spins of the paramagnetic centers to nuclei spins in the sample. As an experimental technique, DNP is well established many decades ago. Its

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potential to neutron science was first demonstrated in neutron diffraction from a lanthanum magnesium nitrate (LMN) crystal [2]. Since then, it has been successfully adapted to scattering from non-crystalline samples [6,7]. However, there exists no DNP equipment today that is suitable for high performance neutron protein crystallography. On the one hand, protein crystals are comparably small and require only compact DNP setups for polarization. On the other hand, the complicated DNP equipment makes it very challenging to provide the large scattering angles required for high performance crystallography. Additionally, high dynamic nuclear polarizations in the past have only been achieved in non-crystalline samples. Further work is still needed for protein crystals, especially in finding more suitable paramagnetic centers required by DNP.

## 2. Initial polarization works at the SNS

Our first effort on polarized neutron protein crystallography from dynamically polarized samples at the Spallation Neutron Source aimed at demonstrating the feasibility of nuclear polarization in protein crystals and building a technical foundation for future applications of polarized neutron protein crystallography [5, 8-10]. The DNP system for this initial effort consisted of a 5 T magnet, a 1 K  $^4\text{He}$  cryostat, and a 140 GHz microwave generator. One of our main research focuses was to achieve a good degree of hydrogen polarization in protein crystals. Selecting a right polarization agent is the first important step to achieving a high degree of nuclear polarization. The exact correlation between the type of agents and the highest achievable nuclear polarization is still under investigation. However, there are a couple of known radicals that have excellent DNP performances for various types of samples. For biological samples in solutions, for example, the Ethyl-hydroxy-butyric-acid Cr(V) complex (EHBA-Cr(V)) [3] and the Tetra-methyl-piperidinyloxy molecules (TEMPO) [11] have both proven to be good options. The EHBA-Cr(V) complex is extensively used in polarized neutron scattering from solution samples [3,5-7, 12,13]. Its downside is that the complex is stable in acidic solutions only. To use with protein crystals, its instability could pose a problem for some samples if it has to be soaked into the crystal for an extended period of time. Therefore, our initial DNP efforts on protein crystals focused on various forms of TEMPO, including those that can act as spin labels, such as the 4-(2-Iodoacetamido)-TEMPO. The 4-(2-Iodoacetamido)-TEMPO can bind to cysteine residues on protein surface. X-rays diffraction data from lysozyme crystals with co-crystallized 4-(2-Iodoacetamido)-TEMPO indicate a high likelihood that TEMPO molecules are bound to the protein. Figure 2 shows some of lysozyme crystal samples with and without the presence of TEMPO [5]. Polarization tests showed that proton polarizations of greater than 30% were readily achievable. While these initial results are promising, these polarization values are too low for protein diffractions and more work is thus needed. In solution scattering, it has

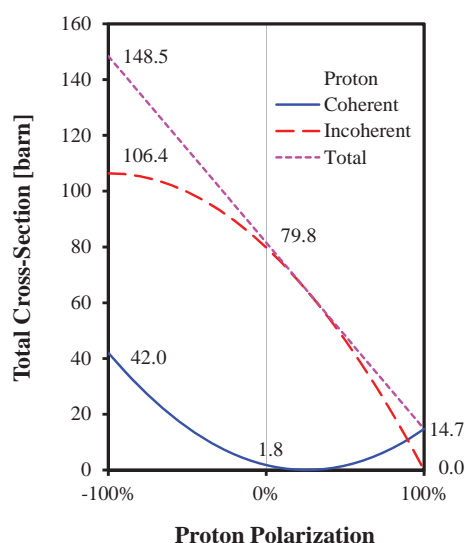


Fig. 1. Coherent and incoherent neutron scattering cross-sections of hydrogen as a function of the proton polarization. Neutron polarization is assumed to be 100%.

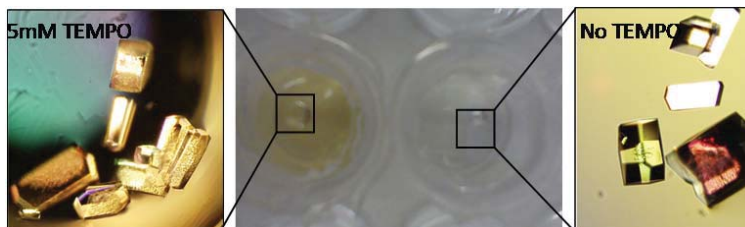


Fig. 2. Lysozyme crystals in 5% NaCl and 50mM Sodium Acetate. Left: with 5mM TEMPO. Right: No TEMPO [5].

been observed that proton polarizations in the bulk and around the paramagnetic centers can be different [7,11]. Such difference can be attributed mainly to two factors. First, the dipole moment of the electronic spin causes the local magnetic field to be different from that of the bulk. Second, the electron spins are presumed to couple with its surrounding nuclear spins only. Nuclear polarization in the bulk is achieved via polarization propagation. In any case, these inhomogeneity in nuclear polarization did not pose a serious problem in solution scattering [3,6,7] due to the low resolution nature of the scattering technique. For protein crystallography, it is unclear whether and how the crystal structure will further influence the distribution of nuclear polarization and affect the diffraction data. For our current effort, while we do not believe polarization inhomogeneity within protein crystals will be our main challenge, we have initiated various studies to investigate the nuclear polarization specific to protein crystals.

### 3. A dedicated DNP setup for protein crystallography

In order to prepare for next generation neutron scattering instruments for the planned second target station at the SNS, we are currently designing and constructing customized DNP sample environment equipment dedicated to neutron protein crystallography. With the huge potential of increasing the coherent signal and decreasing the incoherent signal, we aim at bring the ability to study radically small crystals, protein complexes, and membrane proteins to neutron protein diffractometers at the Oak Ridge National Laboratory.

As mentioned above, dynamic nuclear polarization for scattering typically operates in a magnetic field of 2.5 T – 5 T, which we decided to adhere in our current effort as well. For DNP, field uniformity of better than  $10^{-4}$  over the whole sample is commonly required. This is primarily due to the fact that Bohr magnetons are more than three orders of magnitude larger than nuclear magnetons. A better than  $10^{-4}$  field uniformity will ensure that there will be a clear nuclear Zeeman splitting in the coupled electron-nuclear spin system. Unfortunately, the high field uniformity requirement conflicts with the need of having a large scattering angle in high performance neutron crystallography. For a 2.5 T – 5 T magnet with better than  $10^{-4}$  field uniformity, it is difficult to achieve scattering angles larger than  $\pm 15^\circ$ . The detector on the current neutron protein diffractometer at Oak Ridge covers close to  $\pm 180^\circ$  in azimuthal angle and larger than  $\pm 60^\circ$  in vertical angle. To bridge this gap in scattering angle coverage, we plan to conduct DNP on protein crystals *ex situ*. We have already procured a 5 T, warm bore solenoid magnet and a dilution refrigerator. A customized tail piece will be made for the fridge that incorporates all the needs for the DNP setup. In addition, a small ( $\sim 0.5$  T) super conductor holding magnet will also be built into the tail piece. During the dynamic sample polarization process, the holding field is switched off and the sample-containing tail piece is inserted into the 5 T solenoid magnet. After the polarization, samples will then be cooled down to as close to the fridge base temperature ( $\sim 10$  mK) as possible. After that, the holding field is turned on and the solenoid field is removed. At the low temperature of a few tens of mK, a 0.5 T holding field should be strong enough for a good  $T_1$  nuclear relaxation time and a long neutron data collection time. The solenoid magnet is highly homogeneous and can easily exceed DNP's requirement. The homogeneity of the holding field, on the other hand, can be greatly relaxed. This means that very large scattering angles can be achieved with the holding field. The detail of the holding field coil is still being designed. Our current plan is to have it in the vertical direction and allow a close to  $\pm 180^\circ$  horizontal scattering angle and a  $\pm 50^\circ$  vertical scattering angle.

To continue our research polarization research on protein crystals, we aimed at understanding the connection between the Electron Paramagnetic Resonant (EPR) spectra of the paramagnetic centers and the maximum achievable polarization in protein crystals. Additionally, in protein crystals, crystal orientation may impact the EPR spectra and the maximum achievable nuclear polarization. Gaining such knowledge will help us develop and optimize bulk and site specific spin labelling strategies. In collaboration with various research groups, we have established multiple efforts in this regard.

The commissioning and initial polarized neutron diffraction experiment will be carried out on the IMAGINE instrument [14]. Neutron polarization will be achieved using polarizing supermirrors, which we have tested recently. Our initial experiment will be focusing on well understood proteins such as lysozyme. In the next step, we will move on to more challenging problems such as polymerase involved in DNA repair and photosynthetic antenna complexes.

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